

vapor deposition (CVD), printing, spraying, and other coating methods. For instance, carbon or metal paste based conductive materials are typically formed using screen printing, which either may be done manually or automatically. Likewise, metal-based electrodes are typically formed using standard sputtering or CVD techniques, or by electrochemical plating.

**[0022]** Discrete conductive elements may be deposited to form the detection working electrode **42**, for example, using a patterned mask. Alternatively, a continuous conductive film may be applied to the substrate and then the detection working electrode **42** may be patterned from the film. Patterning techniques for thin films of metal and other materials are well known in the art and include photolithographic techniques. An exemplary technique includes depositing the thin film of conductive material and then depositing a layer of a photoresist over the thin film. Typical photoresists are chemicals, such as organic compounds, that are altered by exposure to light of a particular wavelength or range of wavelengths. Exposure to light makes the photoresist either more or less susceptible to removal by chemical agents. After the layer of photoresist is applied, it is exposed to light, or other electromagnetic radiation, through a mask. Alternatively, the photoresist is patterned under a beam of charged particles, such as electrons. The mask may be a positive or negative mask depending on the nature of the photoresist. The mask includes the desired pattern of working electrodes, which are the electrodes on which the electrocatalytic reactions take place when the detection marker and the redox label are both present and immobilized on the electrode. Once exposed, the portions of the photoresist and the thin film between the working electrode **42** is selectively removed using, for example, standard etching techniques (dry or wet), to leave the isolated working electrode of the array.

**[0023]** The detection working electrode **42** may have a variety of shapes, including, for example, square, rectangular, circular, ovoid, and so forth. For instance, the width (e.g., dimension that is substantially perpendicular to the flow of the test sample) of the electrode **42** may be from about 0.1 to about 10 millimeters, in some embodiments from about 0.5 to about 5 millimeters, and in some embodiments, from about 1 to about 3 millimeters. The surface smoothness and layer thickness of the electrode **42** may also be controlled through a combination of a variety of parameters, such as mesh size, mesh angle, and emulsion thickness when using a printing screen. Emulsion thickness may be varied to adjust wet print thickness. The dried thickness may be slightly less than the wet thickness because of the vaporization of solvents. In some embodiments, for instance, the dried thickness of the printed electrode **42** is less than about 100 microns, in some embodiments less than about 50 microns, in some embodiments less than about 20 microns, in some embodiments less than about 10 microns, and in some embodiments, less than about 5 microns.

**[0024]** In addition, one or more surfaces of the detection working electrode **42** are generally treated with various affinity reagents. Because counter/reference electrode(s) are not disposed on the substrate **80**, the detection working electrode **42** may be advantageously treated with such affinity reagents without fear of contaminating the counter/reference electrode(s). This provides greater freedom in the techniques used to apply the affinity reagents. The affinity

reagents may be applied to the surface of the detection working electrode **42** using a variety of well-known techniques. For example, the reagents may be directly immobilized on the surface of the electrode **42**, may be contained within a substrate that is disposed on the surface of the electrode **42**, may be mixed into the materials used to form the electrode **42**, and so forth. In one embodiment, the affinity reagents are formulated into a solution and screen-printed, ink-jet printed, drop coated, or sprayed onto the working electrode surface. Screen printing inks, for instance, are typically formulated in a buffer solution (e.g., phosphate buffer) containing the specific or non-specific binding members. Although not required, an organic immobilizing solvent may be added to the aqueous buffer solution to help wet the hydrophobic or non-hydrophilic surfaces. In some embodiments, for example, the solvent may be an alcohol, ether, ester, ketone, or combinations thereof. When coated, the electrode **42** is desirably applied with a uniform coating across its entire surface. The coating is typically a single layer, but multiple layers are also contemplated by the present invention. The coating, regardless of monolayer or multiple layers, is typically optimized to give the largest current and signal/noise ratio.

**[0025]** Upon application to the electrode surface, the reagents may optionally be stabilized. Stabilization facilitates long-term stability, particularly for ensuring required shelf-life incurred during shipping and commercial selling. For instance, in one embodiment, stabilization may be accomplished by coating a layer, such as a polymer, gel, carbohydrate, protein, etc., onto the electrode surface before and/or after application of the affinity reagent(s). Some commercially available examples of such a stabilization coating are Stabilcoat®, Stabilguard®, and Stabilzyme® from Surmodics, Inc. of Eden Prairie, Minn.

**[0026]** One example of an affinity reagent that may be applied to the surface of the detection working electrode **42** is a specific binding capture ligand. The specific binding capture ligand is capable of directly or indirectly binding to the analyte of interest. The specific binding capture ligand typically has a specificity for the analyte of interest at concentrations as low as about  $10^{-7}$  moles of the analyte per liter of test sample (moles/liter), in some embodiments as low as about  $10^{-8}$  moles/liter, and in some embodiments, as low as about  $10^{-9}$  moles/liter. For instance, some suitable immunoreactive specific binding capture ligands may include antigens, haptens, aptamers, antibodies, and complexes thereof, including those formed by recombinant DNA methods or peptide synthesis. Generally speaking, electrochemical stability is desired for accurate analyte detection because any redox response from the specific binding capture ligand may complicate the true current responses from the analyte. Thus, in most embodiments, the specific binding capture ligand is stable at the potential range of from -0.75 to +0.75 Volts, in some embodiments from -0.50 to +0.50 Volts, and in some embodiments, from -0.35 to +0.35 Volts, in comparison with the reference electrode.

**[0027]** Besides specific binding capture ligands, redox mediators may also be applied to the surface of the detection working electrode **42**. The redox mediators may be applied to the working electrode **42** at any time, such as during formation of the assay device or during testing. In one embodiment, for instance, the redox mediator is immobilized on the surface of the electrode **42**. Alternatively, the